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A COMPARISON OF THE VISCOSITY AND CERTAIN MICROSCOPICAL PROPERTIES OF SOME KANSAS STARCHES [1]

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INTRODUCTION

The investigation herein reported represents the continuation of an effort to determine the practicability of utilizing Kansas-grown potatoes and sweetpotatoes as starch crops. It has been found previously (*1*)[2] that Kansas potatoes compare favorably with those of other areas in the quantity of the starch they contain. There remained the problem of evaluating them with respect to the quality of the starch.

A partial answer to the practicability of producing starch from the crops of any area is determined by the suitability of the derived starch for nonfood uses, such as pastes, sizings, and mucilages. Accordingly, measurements were made of the physical behavior which may be affected by the granule size, granule size distribution, and the nature of the granule.

The gelatinization temperature is a property of practical importance to starch manufacturers in that it reflects in some measure the suitability of a starch for a given purpose. According to Radley (*11*), there is a close connection between the granule size and the gelatinization temperature, as shown by the fact that, in the same starch suspension, the larger granules appear to swell at a lower temperature than the smaller ones. Furthermore, other factors being equal, starch with an average size smaller than normal will require a somewhat higher temperature and more heat for the conversion to dextrin.

A marked variability is frequently observed in the viscosity of starch pastes at corresponding stages of development. Szego (*14*) and Janicki (*6*) state that the viscosity of a paste is directly related to the average size of the granules, the smaller granules producing a more viscous paste. Caesar and Moore (*3*) have shown that each type of starch will give a characteristic viscosity record, information that is useful in determining the usage of a particular starch. Thus, the individual differences which govern the usefulness of a starch appear to be associated in some way with the permeability and physical structure of the starch granule as well as with the granule size.

The object of this investigation, therefore, was the measurement of the granule size, granule size distribution, gelatinization temperature, and the viscosity of native potato and sweetpotato starches for the purpose of their comparison with each other and with commercial domestic starches and, further, to learn of the effects of time of harvest and cure on the quality of starch.

[1] Received for publication November 5, 1942. Contribution No. 268, Department of Chemistry, Kansas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 345.

EXPERIMENTAL PROCEDURE

TREATMENT OF SAMPLES

The potatoes (*Solanum tuberosum L.*) and sweetpotatoes (*Ipomoea batatas* (*L.*) *Lam.*) used in this investigation were listed and described in an earlier paper (*1*). The samples were obtained from test plots distributed in the potato-growing areas of the State. For their production, proper regard for agronomic procedure was exercised in the selection of soil types, preparation of plot areas, dates of planting and harvesting, cultivation and general care.

Immediately upon arrival of the potatoes, the starch was extracted from all samples except those that were stored or cured, in which cases the starch was not removed until the end of the process. The potatoes were ground in a large meat grinder and sufficient sodium bisulfite was added to give a concentration of 0.15 percent. The ground material was passed through the grinder three additional times, after which the starch was worked out through muslin cloth, washed repeatedly by suspending it in a 0.15-percent soldium bisulfite solution, and allowed to settle before the supernatant liquid was drawn off. After washing the suspension in this manner until the bulk of foreign matter had been removed, and then sieving, the starches were separated by tabling and most of the water was removed on a Büchner funnel under gentle suction. On the following day the starches were air-dried, weighed, and stored in glass containers. Starches thus obtained were white, odorless, and contained practically no foreign matter such as dirt, cellulose, or pigmented granules, that could be detected with a microscope.

DETERMINATION OF THE GELATINIZATION TEMPERATURE

There are several methods of determining the gelatinization temperature. Küntzel and Doehner (*7*) developed a method based on the change in light absorption during gelatinization. Ceasar and Moore (*3*) considered the gelatinization temperature as the range between the temperature at which the viscosity first becomes measurable and that which corresponds to the initial maximum. McNair (*10*), Reichert (*12*), and Francis and Smith (*4*) used that property which the granules possess of losing their polarization crosses when they start to gelatinize. Other methods depend upon the development of a color change in stained granules, a difference in solubility of the granules after gelatinization, and a difference in the volume at the gelatinization temperature. Principally because the gelatinization temperatures of the majority of starches have been based upon the loss of anisotropy, the method of Francis and Smith was selected for this work.

The thermoslide of the gelatinization temperature measuring device was provided with two thermometers for measuring the inlet and outlet temperatures of the heating fluid; the average of these temperatures was taken as the temperature of the starch under observation. The validity of the average temperature was tested with *p*-bromobenzene, whose melting point of 87°–88° C. is not far removed from the temperature range used. The average of several melting-point determinations made on the thermoslide was 87.8°.

In making a determination, two drops of distilled water were placed in the exact center of the upper window, the surface of the water sprinkled with starch, and the dilute suspension covered with a thin cover glass. The microscope was focused upon the suspension and the examination was made with a 4-mm. objective. By the proper adjustment of the polarizer and analyzer with respect to each other, the granules appeared as small bright crosses on a dark background. The fading out of these crosses with the rising temperature marked the disappearance of anisotropy from the granules. The temperature was raised at a rate of 8° C. per minute and the temperatures were taken when the polarization crosses began to fade away regularly and again when nearly all the crosses had faded away. In both potato and sweetpotato starches there were nearly always a few granules the anisotropy of which was lost at an abnormally low temperature and also some whose anisotropy persisted to an extent that, if they were considered, the limits of the gelatinization temperature would be meaningless. Wherever reference is made in the text to the gelatinization and mean gelatinization temperatures, the former is regarded as the temperature range through which the granules lose their anisotropy and the latter as the average of the extreme temperatures of this range.

DETERMINATION OF GRANULE SIZE AND GRANULE SIZE DISTRIBUTION

To determine the granule size of the starch particles, the long axis was measured with a calibrated ocular micrometer on a microscope as described by Garner (*5*). In order to obtain a uniformly dilute sample to observe, a small quantity of starch was placed in two drops of glycerol on a black glass plate and mixed thoroughly; a very small portion of this was placed on the slide with the addition of another drop of glycerol and covered with a cover glass. In this manner 500 granules from a uniformly dilute mixture were measured. To check upon the reliability of this method a count of 5,000 granules was made from a sample, and the results showed that a count of 500 was representative. Lindet and Nottin (*9*) state that a starch can be characterized with sufficient accuracy if 150 to 200 granules are measured, but it was found in this study that a count of less than 500 does not always permit a satisfactory statistical treatment.

DETERMINATION OF VISCOSITY

The viscosity of the starch pastes was measured in a viscometer designed by Barham and Reed; the description and calibration of the instrument, together with the detailed method of making determinations, are given by Barham, Wagoner, and Reed (*2*).

The viscosity is measured by the weight in grams that must be applied to prevent the rotation of a cylinder suspended in a rotating cup containing the starch paste. This relationship is defined by the equation

$$\frac{W}{\eta l}=k$$

in which W represents the weight in grams, η the absolute viscosity. l the height of the cylinder wall exposed to the action of the liquid, and k the instrument constant which in this case has a value of 0.79,

In making determinations, the starches were made into 10-percent "starch milks" which were heated slowly to 93°–94° C. After cooking the resulting pastes at 93° for one-half hour, they were cooled slowly to 35°–40°. Throughout the temperature cycle, temperature and viscosity readings were made at regular intervals.

RESULTS

VISCOSITY

Viscosity determinations were made on all samples prepared in the laboratory, and table 1 gives a representative sample of the data

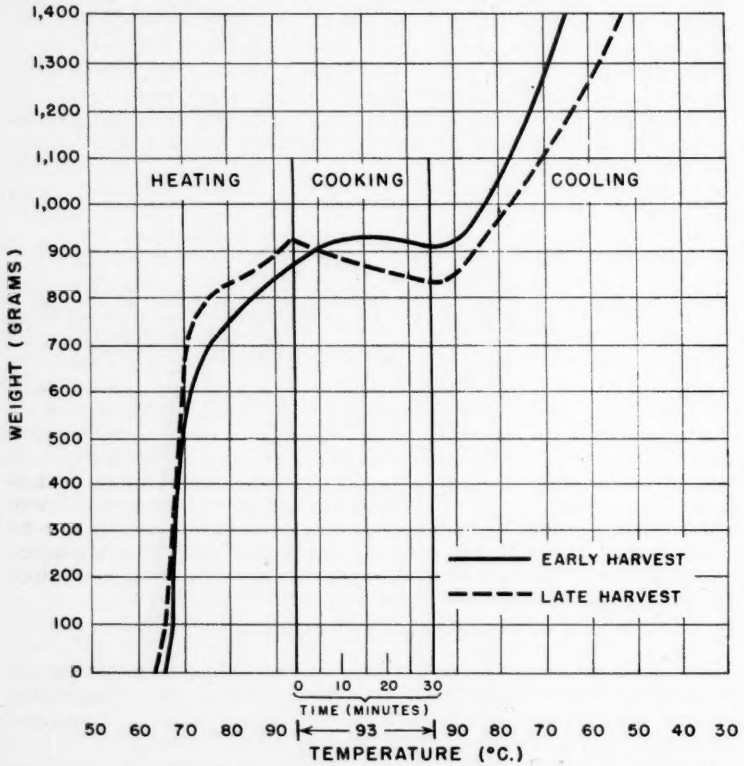

FIGURE 1.—The effect of time of harvest on the viscosity of the starch from potatoes (Warba variety).

taken. In each determination data were taken each minute until the cooking period was reached after which readings were taken at 5-minute intervals; but to shorten the table the data are listed for only 5-minute intervals. From such data smooth curves result when the weight in grams (W), as measured by the balance, is plotted against the temperature.

The effects of early and late harvest upon the starch from the Warba variety of potatoes are shown in figure 1. The curves in this figure are representative of all three varieties tested; in every case the late-harvest potatoes formed a starch that had a lower temperature at the first observable change in viscosity, thinned out more dur-

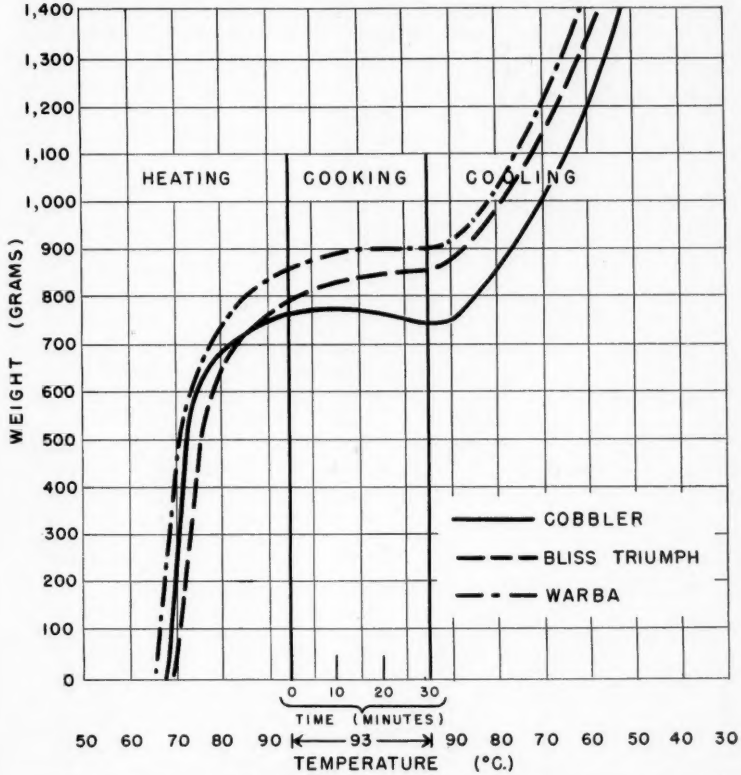

FIGURE 2.—Comparison of the viscosity of starch from three common varieties of potato, indicating the effect of variety.

ing the cooking, and never attained the maximum viscosity of the starch from early potatoes.

In figure 2 there is a comparison of starches of the three common varieties of potatoes. This shows that the Warba exhibited the lowest temperature at which the viscosity curve starts, the highest maximum, and the most viscous paste when cooled. The Bliss Triumph showed a higher temperature than Irish Cobbler for the initial part of the viscosity curve, but the Cobbler thinned out more on cooking and did not give so viscous a paste on cooling.

TABLE 1.—*Viscosity record of a 10-percent suspension of late-harvest Bliss Triumph (sample 15)*

Time	Oil-bath temperature	Paste temperature	Temperature lag	Weight (*W*)	Time	Oil-bath temperature	Paste temperature	Temperature lag	Weight (*W*)
	°*C.*	°*C.*	°*C.*	*Grams*		°*C.*	°*C.*	°*C.*	*Grams*
1:30	69.0	61.0	8.0	------	2:40	97.0	92.0	5.0	803
1:35	70.5	65.0	5.5	------	2:45	98.0	93.0	5.0	798
1:40	73.0	67.7	5.3	------	2:50	97.0	92.7	4.3	795
1:45	75.5	70.5	5.0	20	2:55	97.0	92.2	4.8	795
1:50	78.0	71.7	6.3	255	3:00	88.0	88.0	0	830
1:55	80.0	73.5	6.5	535	3:05	74.0	80.0	6.0	940
2:00	81.0	76.0	5.0	612	3:10	70.0	76.0	6.0	990
2:05	83.5	77.7	5.8	626	3:15	64.0	71.0	7.0	1,090
2:10	86.0	80.5	5.5	654	3:20	60.0	66.0	6.0	1,190
2:15	92.0	84.0	7.0	680	3:25	54.0	61.0	7.0	1,295
2:20	96.0	89.0	7.0	703	3:30	48.0	56.0	8.0	1,440
2:25	98.0	92.0	6.0	750	3:35	43.0	49.0	6.0	1,610
2:30	97.0	92.5	4.5	790	3:40	39.0	45.0	6.0	1,750
2:35	96.0	92.0	4.0	810					

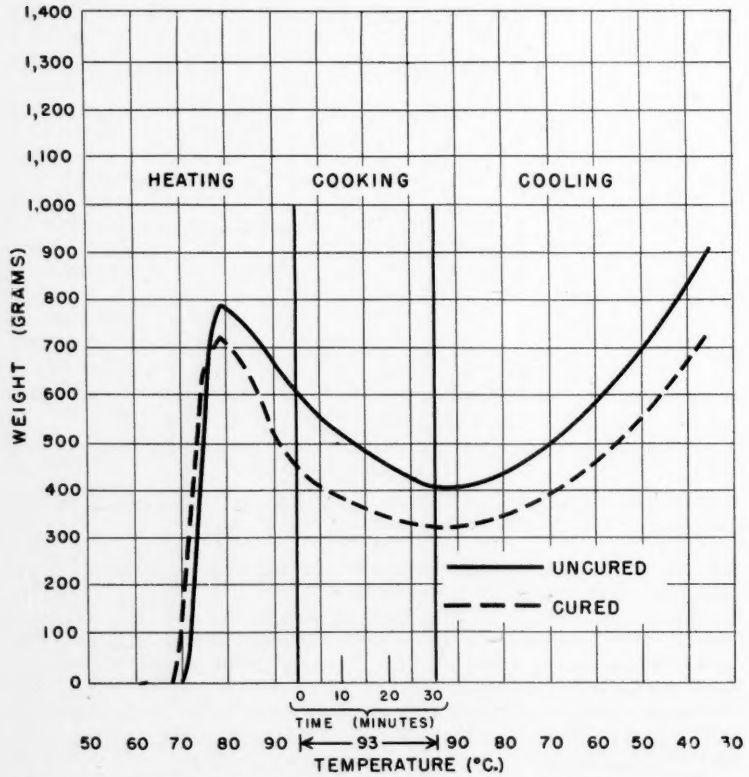

FIGURE 3.—The effect of curing on the viscosity of starch from sweetpotatoes.

Figure 3 represents the effect of curing on the sweetpotato starches. it is to be noted from figure 3, which is typical of all the starches examined, that the starch from the cured sweetpotatoes had different properties from that of the uncured. This starch showed the initial viscosity change at a lower temperature, did not become so viscous, and thinned out more than the starch from the uncured sweetpotatoes. Figure 4 is a viscosity graph of the five varieties of sweetpotatoes which, with the exception of the Big Stem Jersey variety, were grown in the same plots and under similar conditions. The Little Stem

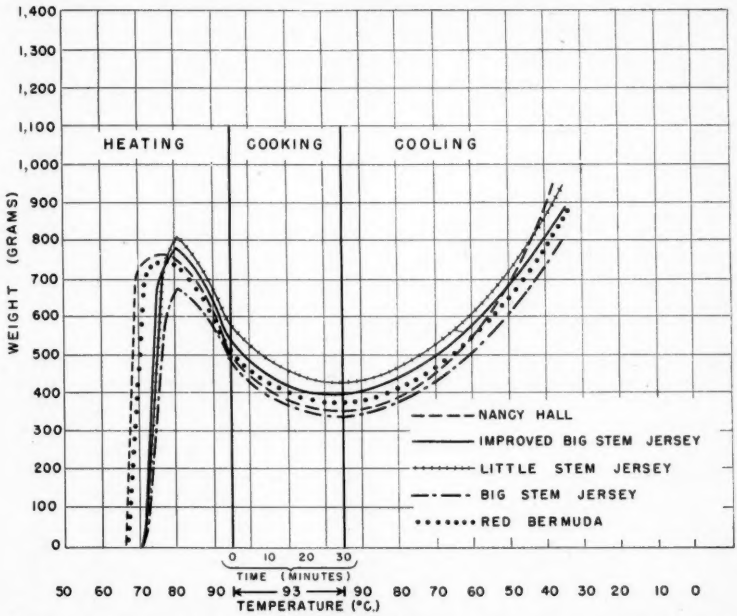

FIGURE 4.—Comparison of the viscosity of starch from five varieties of sweetpotatoes, indicating the effect of variety.

Jersey formed the most viscous paste with the least thinning out, while the Big Stem Jersey formed the least viscous paste with the most thinning out. In general the shapes of the viscosity curves of the five varieties are about the same, although viscosity of the Nancy Hall and Red Bermuda varieties developed at lower temperatures and continued over a wider temperature range during the heating stage.

GELATINIZATION TEMPERATURE AND AVERAGE DIAMETER OF GRANULES

The results of the gelatinization temperature and the average diameter measurements of starches are given in tables 2 and 3. Usually in the literature only one temperature is given for sweetpotato or potato starch, and more frequently no mention is made of the particular variety upon which the determination was made.

But in this study it is shown that there were varietal and environmental variations with respect to gelatinization temperature and granule size for potatoes and sweetpotatoes. The variation in the extremes of the gelatinization temperature was from 4.5°–6.0° C. for potato and 5.5°–10.0° for sweetpotato starches. A statistical analysis [3] of these data shows that there are significant varietal differences in the gelatinization temperature.

TABLE 2.—*Effects of variety and curing on the gelatinization temperature, mean gelatinization temperature, and average granule diameter of sweetpotato starches, and a comparison of these starches with starch from a commercial source*

Sample No.	Source of starch	Treatment	Gelatinization temperature	Mean gelatinization temperature	Average diameter of granules
			°C.	°C.	*Microns*
5	Little Stem Jersey	None	70.5–77.0	74.0	9.10
16	do	Cured	68.5–77.5	73.0	8.27
12	Red Bermuda	None	66.5–76.5	71.5	9.50
14	do	Cured	66.0–72.5	69.0	8.96
6	Improved Big Stem Jersey	None	70.0–79.5	75.0	9.78
15	do	Cured	68.0–78.0	73.0	8.69
10	Big Stem Jersey	None	70.5–80.5	75.5	9.12
13	Nancy Hall	None	67.0–72.5	70.0	8.49
(1)	Stein, Hall & Co		70.0–76.0	73.0	11.44

[1] Domestic commercial starch included for comparison.

TABLE 3.—*Effects of variety and time of harvest on the gelatinization temperature, mean gelatinization temperature, and average granule diameter of potato starches, and a comparison of these starches with starch from a commercial source*

Sample No.	Source of starch	Time of harvest	Gelatinization temperature	Mean gelatinization temperature	Average diameter of granules
			°C.	°C.	*Microns*
1	Irish Cobbler	Early	67.0–73.0	70.0	23.67
8	do	Late	69.0–75.0	72.0	26.06
4	do	Early	66.5–72.5	69.5	24.26
16	do	Late	67.0–73.0	70.0	26.08
2	Bliss Triumph	Early	66.5–71.5	69.0	22.64
15	do	Late	70.0–75.0	72.5	22.40
3	Warba	Early	70.0–74.5	72.5	33.91
14	do	Late	69.5–75.0	72.5	28.28
(1)	Stein, Hall & Co		59.5–68.5	64.0	29.06

[1] Domestic commercial starch included for comparison.

Table 2 also illustrates the effect of curing upon the gelatinization temperature and granule size. In the curing process, which is done to heal the breaks in sweetpotatoes and to give a sweeter taste, the sweetpotatoes were heated at 26.7°–32.2° C. for 10 days to 2 weeks. The curing had the effect of lowering the gelatinization temperature and reducing the granule size.

Table 3 summarizes data showing the effects of the time of harvest upon the gelatinization temperature and the granule size of potato starches. These data indicate that the later harvest raised the gelatinization temperature for the Irish Cobbler and Bliss Triumph

[3] The statistical analysis was made under the supervision of Dr. H. C. Fryer, Department of Mathematics, Kansas State College.

(for Warba there was no change), raised the average granule size for the Irish Cobbler, and lowered the average granule size for the Warba and Bliss Triumph.

GRANULE SIZE DISTRIBUTION

When the granule sizes of the starches were arranged as to their frequency, it was found that both the potato and the sweetpotato starches were quite regular, with a majority of the granules about halfway between the two limits. Table 4 gives a representative sample of the granule size and distribution for a potato starch. In table 5, the percentage of granules occurring in each size range is given for the sweetpotato starches; similar data are given in table 6 for potato starches. It is evident that the effects of variety and curing on sweetpotato starches and of variety and time of harvest on potato starches showed different ranges and distribution of sizes.

TABLE 4.—*Data obtained in the size-frequency determinations of potato starch (sample 1)*

Step interval (microns)	Midpoint	Frequency		Step interval (microns)	Midpoint	Frequency	
	Microns	*Number* [1]	*Percent*		*Microns*	*Number* [1]	*Percent*
2–6	4	0	0	38–42	40	23	4.6
6–10	8	24	4.8	42–46	44	15	3.0
10–14	12	64	12.8	46–50	48	13	2.6
14–18	16	87	17.4	50–54	52	3	.6
18–22	20	102	20.4	54–58	56	5	1.0
22–26	24	67	13.4	58–62	60	3	.6
26–30	28	40	8.0	62–66	64	1	.2
30–34	32	27	5.4	66–70	68	1	.2
34–38	36	25	4.0	70–74	72	0	0

[1] Total=500.

TABLE 5.—*The percentage of granules occurring in each size range for some sweetpotato starches*

Midpoint (microns)	Improved Big Stem Jersey		Little Stem Jersey, uncured	Big Stem Jersey, uncured	Red Bermuda uncured	Nancy Hall, uncured
	Uncured	Cured				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0						
2		0.4			1.0	2.6
4	7.2	8.8	7.0	4.8	8.4	6.8
6	15.8	18.6	18.0	19.2	11.4	21.8
8	25.0	40.8	30.6	20.8	32.4	34.4
10	19.8	10.0	21.0	21.6	17.4	16.4
12	15.4	12.6	11.4	14.6	15.4	10.8
14	7.0	4.0	6.6	4.4	5.6	3.0
16	5.8	2.8	3.8	2.8	4.2	2.0
18	1.4	.2	1.2	1.2	2.0	.6
20	1.8	1.0	.4	.4	1.8	1.6
22	.2	0		.2	.2	
24	.2	.6				
26	.4	.2				
28						
30						
Average diameter	*Microns* 9.78	*Microns* 8.69	*Microns* 9.10	*Microns* 9.12	*Microns* 9.50	*Microns* 8.49

TABLE 6.—*The percentage of granules occurring in each size range for some potato starches*

Midpoint (microns)	Warba (early harvest)	Late harvest			Midpoint (microns)	Warba (early harvest)	Late harvest		
		Warba	Bliss Triumph	Irish Cobbler			Warba	Bliss Triumph	Irish Cobbler
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
0					48	6.8	3.0	1.2	2.2
4					52	4.8	3.4	1.6	1.0
8	1.0	4.6	6.2	5.0	56	3.0	2.0	.2	1.0
12	3.8	9.0	19.0	7.6	60	2.4	2.2	.6	1.8
16	5.0	12.2	15.8	12.2	64	2.2	.2	.2	.2
20	10.6	16.6	16.8	15.6	68	.6	.6		.2
24	10.8	8.8	9.8	17.4	72	.8			.2
28	13.8	8.4	10.4	12.4					
32	10.2	8.0	9.0	9.8					
36	10.2	6.8	3.6	5.6					
40	9.2	8.6	4.4	4.6	Average	*Microns*	*Microns*	*Microns*	*Microns*
44	4.8	5.6	1.2	3.2	diameter	33.91	28.28	22.40	26.06

DISCUSSION

Measurements of the viscosity, the granule size, and granule size distribution, and the gelatinization temperatures show that varietal and environmental factors affect the properties of the starch in some manner. It is recognized, however, that the environmental factors in these experiments were restricted both in extent and kind, and interpretations of subsequent comments should be made subject to these limitations. If conditions should prevail in which other factors would be imposed or the same factors would have greater or lesser importance, it is assumed that the results obtained would no longer be valid.

In earlier paragraphs, stress was placed upon the probable importance of granule structure in the pasting characteristics of starches. As the term "structure" was used, it was intended to include every chemical individual which contributed to the make-up of the granule. Küntzel and Doehner (*8*) have discussed the part played by granule permeability in pasting behavior. It is to be presumed that the permeability may be partly determined by the nature and amounts of adsorbed substances which, in turn, might well vary because of varietal and environmental differences. If this is true, the nature and amounts of adsorbed materials, as well as the granule size and granule size distribution, would affect the viscosity records and gelatinization temperatures of starches from a given source.

VARIETY COMPARISON, POTATOES

When the starches from three varieties of potatoes were compared, it was found that the starch from Warba exhibited the highest gelatinization temperature, the largest granule size, and formed the most viscous paste which thinned out least during cooking. The Bliss Triumph formed a starch paste that had the lowest gelatinization temperature and the smallest granule size, but the maximum viscosity was slightly higher than that of the Irish Cobbler. From this it may be concluded that the varietal factor makes more difference in the viscosity of a paste formed from starch than the granule size or granule size distribution, and that the viscosity is not always reflected in the gelatinization temperature. This may be accounted for by

assuming differences in permeability or internal physical structure, or both.

Of the varieties tested, the Warba produced the best starch for a very viscous paste with the least thinning out when cooked.

VARIETY COMPARISONS, SWEETPOTATOES

When the viscosity examinations were made of the sweetpotato starches, it was found that the viscosity curves of Red Bermuda and Nancy Hall were quite similar in form to those of the Jersey varieties, but the latter curves started 5° to 6° C. higher. The same difference was noticed in the gelatinization temperatures, but the granule sizes were about the same. A comparison of their viscosity showed that the Little Stem Jersey formed the most viscous paste and the one that thinned out the least, while the Big Stem Jersey formed a paste of the lowest viscosity. It may be concluded, therefore, that the varietal factor of sweetpotatoes will make more difference in the quality of the starch obtained than the granule size or size distribution.

Of the varieties tested, the Little Stem Jersey formed a starch that produced the most viscous paste and the paste that thinned out the least.

EFFECT OF CURING UPON STARCH FROM SWEETPOTATOES

Both the cured and uncured samples were taken from the same lot of sweetpotatoes. It was observed that curing caused similar changes in the properties of the starches obtained from all varieties. Its effect was to lower the gelatinization temperature, give the starch granules a smaller average size, and materially decrease the viscosity of the starch pastes. If it may be accepted that, other things being equal, the gelatinization temperature (*11*) and the viscosity (*14, 6*) are higher the smaller the granule diameter, it follows that curing, by lowering the gelatinization temperature and viscosity with a decrease in granule size, modified the structure of the starch granule.

EFFECT OF TIME OF HARVEST UPON POTATOES

The varieties of potatoes harvested at different periods were the Bliss Triumph, Warba, and Irish Cobbler. It was found that at late harvest, the gelatinization temperature of the starch was higher (except for the Warba, which did not change) and both the viscosity and the temperature of the initial development of viscosity were lower. But the effect of late harvest upon the granule size varied with the variety; granules of the Cobbler became larger, those of Bliss Triumph remained the same, and those of the Warba became smaller at late harvest. It accordingly is apparent that alteration of the behavior of starch due to the time of harvest is not predictable on the basis of changes in granule size. Here, as in the case of sweetpotato cure, there is the suggestion that the granules have undergone structural modification.

COMPARISON OF POTATO AND SWEETPOTATO STARCHES

Native potato and sweetpotato starches differed from each other in almost every respect. The gelatinization temperature of the potato starch was lower, the average granule size was much larger, and the

size distribution of the granules was over a wider range than the sweetpotato starches. But the greatest difference between them was to be found in their viscosities. The general forms of the two viscosity curves were different; sweetpotato starch formed a paste that became quite viscous within a few degrees above the lower limit of the gelatinization temperature, began to thin out immediately on reaching the upper limit, and when cooled was never highly viscous. Potato starch always began its viscosity curve below the lower limit of the gelatinization temperature, developed its maximum viscosity more slowly, but after it was attained, did not thin out to any great extent. Both starches formed pastes which were clear. From these observations it appears that native potato starch would possess excellent quality for any purpose requiring a paste of high viscosity, whereas sweetpotato starch should serve for purposes which require a thinner paste.

It was observed that the viscosity curves of the potato starches started below the lower limit of the gelatinization temperatures and continued after the upper limit was reached while those for sweetpotato starches always began at the lower limit and began to thin out as soon as the upper limit was reached. From this it may be inferred that potato starches swell a great deal below and above the gelatinization temperature, while in sweetpotato starches there must be very little, if any, swelling before the granules begin to lose their anisotropy or after anisotropy has disappeared. It follows, therefore, that the gelatinization temperature, based on the loss of anisotropy, does not necessarily correspond to that part of the viscosity curve between zero viscosity and the initial maximum.

COMPARISON OF NATIVE WITH DOMESTIC COMMERCIAL STARCHES

The study of the quality of native potato and sweetpotato starches would not be adequate without a comparison with domestic commercial starches. Starches obtained from Stein, Hall & Co. were used for this purpose.

Figure 5 provides a comparison of the viscosity of a Little Stem Jersey starch with the commercial sweetpotato starch; table 2 shows how the commercial starch compares with native starches with respect to gelatinization temperatures and granule size. It is apparent that the commercial starch, except for its appreciably larger granule size, closely resembles that obtained from the Jersey varieties.

Native potato starch, on the other hand, differs markedly from domestic commercial starch except in granule size. The gelatinization temperature of the latter is considerably lower than that of any of the native starches (table 3) and its viscosity record, although much higher during the heating stage, is similar in general form (fig. 6) to that of sweetpotato starch (fig. 5).

It was only after a series of experiments on the effect of cure on the quality of sweetpotato starches[4] that a satisfactory explanation was found for the marked difference between native potato starches and the commercial starch used. In these experiments results were obtained which strongly indicate that pasting behavior is partly determined by the extensiveness of adsorption throughout the starch granule and the nature of the materials adsorbed. Küntzel and

[4] BARHAM, H. N., and WAGONER, J. A. Unpublished data.

Doehner (*7*), under similar circumstances, had ascribed differences in pasting behavior to impurities which had not been removed, but no supporting experimental evidence was given.

In order to determine whether this marked difference in pasting behavior was due to adsorbed substances, the commercial starch was exhaustively extracted with 85-percent methanol according to the method of Schoch (*13*). The viscosity records of both the extracted and unextracted commercial starch together with a native starch (late-harvest Warba) are shown in figure 6. These results indicate

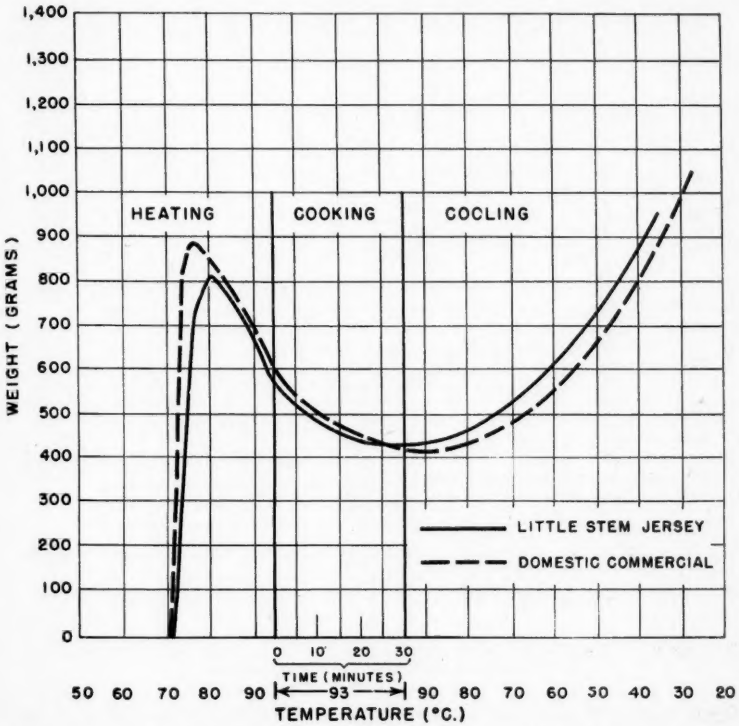

FIGURE 5.—Viscosity curve for native Little Stem Jersey sweetpotato starch compared with that for a domestic commercial sweetpotato starch.

that extraction has had the effect of changing the quality of the extracted starch to the extent that it possesses the same general pasting behavior as that of native starches.

It is not to be concluded, however, that the native starches were of necessity purer. Similar extractions were made also of native potato and of native and commercial sweetpotato starches. Although there were some changes in the viscosity records as a consequence of extraction, the general forms of the curves remained unaltered. Although no conclusions may be drawn from these experiments as to the significance of the amounts of materials adsorbed, the nature of such materials

does appear to have an important bearing on pasting behavior. A discussion of the origin of differences in the nature of the adsorbed materials on potato starches would involve only speculation; there are numerous ways in which such differences could resaonably be accounted for.

CONCLUSIONS

Varietal and environmental differences in potatoes and sweetpotatoes were reflected in viscosity, gelatinization temperature, granule size, and granule size frequency measurements of the starches obtained

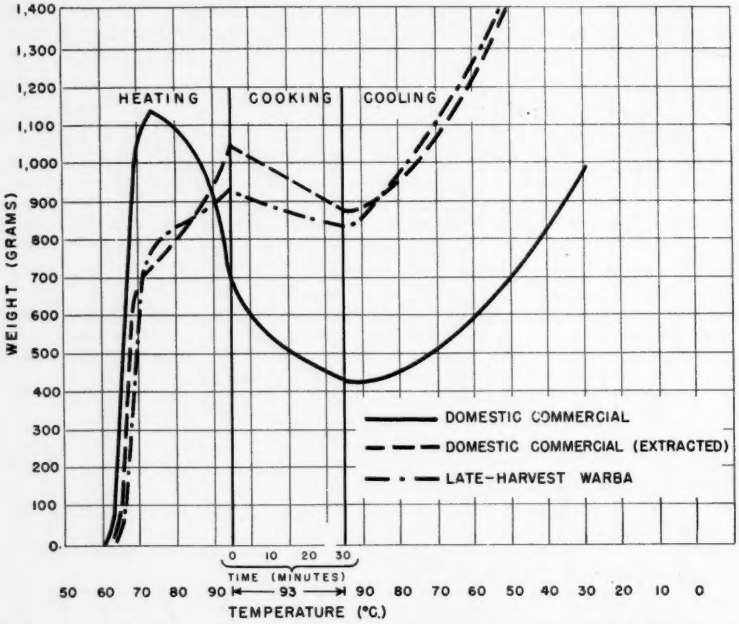

FIGURE 6.—Viscosity curve for native potato starch compared with that for a domestic commercial potato starch.

from them. There was no direct correlation of the granule size and granule size frequencies, as they were affected by such differences, with either the viscosity or the gelatinization temperature, nor were the gelatinization temperature and viscosity directly related. It may be assumed that varietal and environmental factors in some manner affect the structure of the starch granule. That structural differences due to adsorbed materials are capable of markedly affecting the pasting behavior of a starch has been demonstrated.

The gelatinization temperature, based on the loss of anisotropy, does not necessarily correspond to that part of the viscosity curve between zero viscosity and the initial maximum.

Five hundred granules seem to be a sufficient number to count in order to obtain the average size and the size frequency. A smaller number than this does not always permit a reliable statistical treatment.

Curing of the sweetpotatoes lowered the gelatinization temperature, the granule size, and the viscosity of the starches, indicating a change in the nature of the granule during the curing.

The late harvest of potatoes tended to raise the gelatinization temperature and to decrease the viscosity, but the average size of the granules was either smaller or larger, depending upon the variety.

The Warba variety of potatoes and the Little Stem Jersey variety of sweetpotatoes formed the most viscous pastes and the ones that thinned out least during cooking.

Starches obtained from Kansas potatoes and sweetpotatoes are of good quality and are the equal of commercial starches if, in the case of potato starch, the basis for comparison is extracted starch.

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EFFECT OF CORN BARRIERS ON NATURAL CROSSING IN COTTON [1]

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INTRODUCTION

Natural crossing has important implications throughout the cotton industry, from the technique of breeding superior strains to the final utilization of manufactured products. Segregation from uncontrolled hybridization may cause adverse effects in any stage of cotton breeding, production, or utilization in which fiber quality or uniformity is concerned. The quality of cotton lint is dependent on many environmental and genetic factors. Environmental factors, including soil uniformity, fertilization, and cultural practices, are subject to some control; but in the greater part of the Cotton Belt, moisture supply is dependent on rainfall, and since this important factor is not subject to control, the entire complex of ecological factors and responses becomes largely fortuitous. In contrast, the genetic constitution, which determines potential fiber properties, plant characteristics, and yield, may be fixed through careful breeding and protected from contamination in the various stages of multiplication, distribution, and production.

The diversity of plant and fiber characters exhibited by commercial varieties of cotton is due largely to segregation following natural cross-pollination between unlike plants. All types of cotton grown commercially in the United States hybridize readily, and rapid mongrelization occurs when unlike types, varieties, or strains are planted in proximity, unless planting seed from the several lots is protected from natural crossing.

In breeding work with cotton, genetic purity is of major importance in developing superior strains. Breeders may conveniently control pollination in establishing and maintaining purity during the various stages of selection, but when small stocks are increased to multiplication-block or field size, artificial self-pollination becomes too costly, and isolated multiplication of the stocks must be used if mixture is to be prevented. The distance required for adequate isolation and the extent of cross-pollination between close or adjacent plantings of unlike cottons have been studied by numerous investigators. The present study was undertaken to furnish information on the effectiveness of guard rows of corn in protecting cotton multiplication blocks from natural crossing.

[1] Received for publication March 18, 1943.

LITERATURE REVIEW

Comprehensive reviews of literature on this subject have been given by Kearney (*5, 6*),[2] Brown (*3*), and others. Kearney (*5, p. 11*), in summarizing the results of his own experiments and those of other investigators, states:

> * * * The proportion of vicinists rarely exceeds 20 per cent, however, and is usually much smaller. The available information in regard to vicinism therefore points strongly to the conclusion that in cotton self-fertilization greatly predominates over cross-fertilization. It should not be inferred, however, that because most of the ovules normally are self-fertilized, such cross-fertilization as occurs is negligible in its effect upon the uniformity of a variety.
>
> As a rule, the percentage of vicinists decreases rapidly as the distance between the seed-bearing and the pollen-bearing parents increases, but the data at hand do not permit a conclusion to be drawn as to the degree of isolation necessary to eliminate the danger of cross-pollination. This is doubtless affected by the nature of the varieties grown, by local and seasonal variations in the insect population and in the flowering of other plants, and by topography, weather, and other factors.

Ware (*12*) concluded that the amount of natural crossing in cotton varies with the season and locality and is determined by the number of insects present and capable of carrying pollen from one flower to another. He also called attention to the fact that much natural crossing is never detected, since it can be observed only when the pollen is carried from a plant that possesses a heritable detectable difference.

From experiments conducted at State College, Miss., in 1918, Brown (*3*) reported 3.9 to 5.6 percent of crossing on rows of green-leaf cotton growing next to red-leaf cotton, less than 1 percent on the 2d to 8th rows from the red-leaf, and no crossing on rows 9 to 12. In 1919, however, with a larger amount of red-leaf cotton (27 rows) planted at one end of a field of green-leaf, 14.8 percent hybrids were obtained from seed of the row next to the red-leaf, 3.2 percent on the 5th row, 1.9 percent on the 10th, and from 0.6 percent on the 16th to 0.06 percent on the 114th row. There were 119 rows in the field. Brown attributed the larger amount of crossing in 1919 to the larger number of red-leaf plants included in the experiment. Richmond et al. (*9*) reported an average of 9 percent of natural crossing between upland and sea-island stocks from experiments conducted in Texas. Allard (*1*), Balls (*2*), McLendon (*7*), the Mississippi station (*8*), Shoemaker (*10*), Stroman and Mahoney (*11*), Webber (*13*), and others have reported amounts of natural crossing observed ranging from less than 1 to more than 60 percent.

It is evident from the literature that climatic and biotic factors greatly influence the amount of natural crossing. Although serious consideration has been given to the effect of insect populations and distances between plantings of unlike parents, an equal abundance of unlike pollen has been lacking in many previous studies. Equality in amount of pollen would be most nearly approximated with equal areas of unlike plants. Many previous experiments have consisted of single rows or small plots of red-leaf plants entirely surrounded by green-leaf plants. Data have been given showing the extent of crossing in rows of green-leaf cotton adjacent to red-leaf and in rows of green-leaf removed by 5, 10, 20, or more intervening rows from red-leaf cotton. Data of this nature must be interpreted with care, insofar

[2] Italic numbers in parentheses refer to Literature Cited, p. 360.

as application to practical problems of increase is concerned, since the amount of pollen available from normal plants was, in many of the reports cited, considerably greater than that from the contrasting genotype.

METHOD OF INVESTIGATION

In breeding work it usually is necessary to plant numerous increase blocks of different strains for the multiplication of seed from superior progenies. Complete isolation is frequently difficult to arrange, and it has been a common practice to plant multiplication blocks within short distances of each other with or without intervening rows of corn or other tall-growing crops. On the basis of previous information, such practices might be assumed to afford a fair degree of isolation.

Experiments on natural crossing in cotton were conducted at Knoxville, Tenn., in 1939–40 and 1940–41 to test the practicability of corn barriers in protecting unlike cottons from natural crossing. Upland cottons (*Gossypium hirsutum* L.) possessing red-plant and green-plant colors were chosen for the unlike parents, since the intermediate inheritance of plant color (*12*) provides a convenient means of identifying the hybrids. Equal areas of the contrasting types were included in the experiments in order that the study might be representative of practical field requirements and might also provide a simple basis for comparison.

The plan of planting in each season provided four test conditions: (1) Green adjacent to red but guarded from any other cotton by corn barriers; (2) green separated from red by three rows of corn; (3) green separated from red by six rows of corn; and (4) green separated from red by nine rows of corn (fig. 1). In 1939 four-row plots were used, but owing to limited space in 1940 three-row plots were used for both green and red genotypes. The four replications of each test condition were arranged in a Latin square to reduce the effect of external factors, such as the direction of prevailing winds, insect habitats, and adjoining crops.

The plots of red-plant and green-plant cotton and the corn barriers were planted on the same date. Flowering in the two kinds of cotton covered approximately the same period, but the green variety was somewhat more prolific than the red, especially in the 1940 field planting. In both seasons the corn barriers reached a height of 8 to 10 feet and were well advanced in growth before the cotton began to flower.

Seed cotton was harvested and ginned by rows from all plots of green plants, and the seed from each row was planted the following spring in a corresponding 100-foot row in order that the amount of hydridization might be measured by the proportion of intermediate red plants. The total number of plants thus grown was 86,951, from the 1939–40 experiment, and 50,256, from the 1940–41 experiment. Records were made of the total number of plants and hybrids in each row, and from these data the percentage of hybrids was calculated.

In 1941 the scope of the study was extended to cover the effect of isolation distances, ranging from approximately 0.1 to 0.8 mile, on natural crossing. Small blocks of pure red-leaf cotton were grown at six places surrounding the station; the open-pollinated seed produced

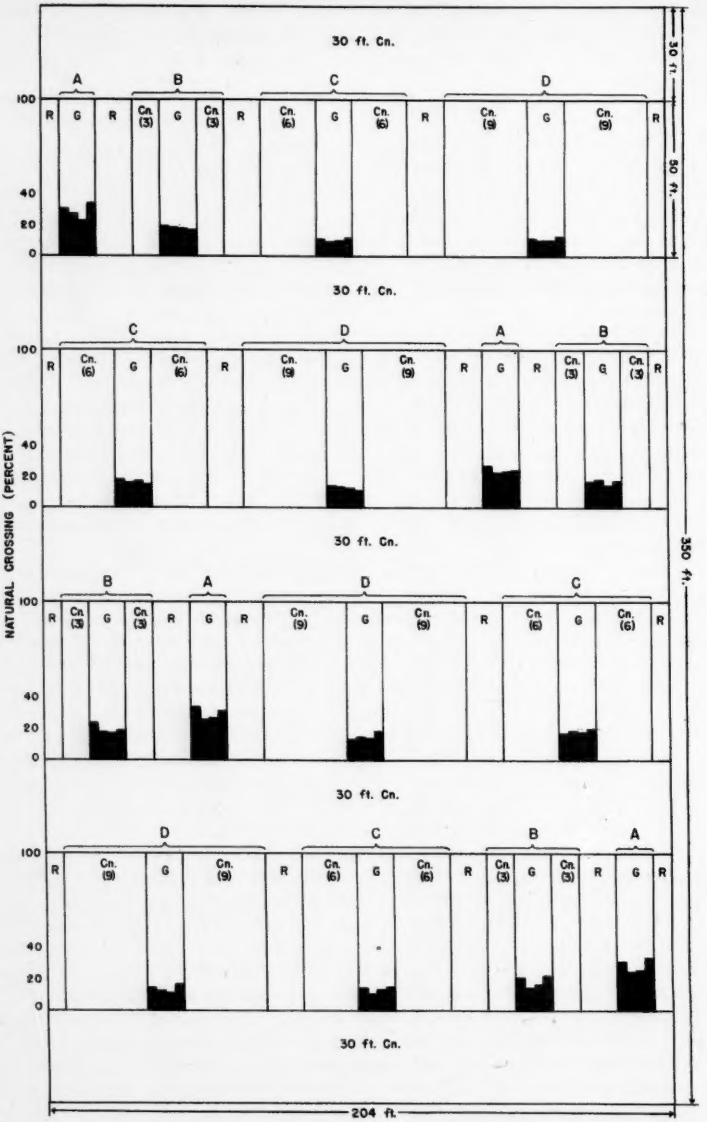

FIGURE 1.—Field plan of natural-crossing experiment in 1939, showing location of green-leaf cotton, red-leaf cotton, and corn barriers. *G* indicates green-leaf cotton, *R* red-leaf cotton, and *Cn.* corn. The numbers in parentheses indicate the number of rows in each corn barrier. The four vertical bars in each green-leaf plot show the percentage of natural hybrids by rows.

in each of the blocks was planted in 1942; and the resulting plants were classified to determine the number of natural hybrids. In this part of the study no artificial barriers were used, so that protection from natural crossing depended on distance, topography, and natural barriers of trees, brush, or intervening fields of other crops or of grassland.

ANALYSIS OF DATA

Data from the 1939–40 study on the total number of plants and the percentage of red plants are shown by plots and rows in table 1. For convenience in comparing the data, the 4 replicates for no barrier, 3-row barrier, 6-row barrier, and 9-row barrier of corn, respectively, are grouped together. The total plant count and the percentage of natural crosses are shown by rows and plots for the reason that in the comprehensive analysis it seemed desirable to compare the percentage of natural hybrids in the outside and inside rows of the various plots. The total number of plants per row, though in all cases adequate for a satisfactory percentage determination, ranged from approximately 1,050 to more than 1,600; consequently, percentages as computed from the plot totals and as averaged for separate rows differed slightly. Under these circumstances, a simple analysis of variance based on percentages calculated from plot totals will not coincide exactly with the whole-plot section in the comprehensive analysis of variance, in

TABLE 1.—*Total number of plants and the percentage of red plants by plots and rows, natural crossing study, Knoxville, Tenn., 1939–40*

No barrier			3-row corn barrier			6-row corn barrier			9-row corn barrier		
Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants	Plot and row	Total plants	Intermediate red plants
	Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*		*Number*	*Percent*
I–A–1	1,414	30.2	I–B–1	1,417	19.2	–C–1	1,498	11.1	I–D–1	1,527	11.9
2	1,489	27.1	2	1,353	18.0	2	1,299	9.1	2	1,160	10.8
3	1,566	22.5	3	1,463	17.0	3	1,515	9.4	3	1,274	10.8
4	1,488	33.0	4	1,291	16.7	4	1,425	11.7	4	1,431	12.6
Total	5,957	28.1	Total	5,524	17.7	Total	5,737	10.4	Total	5,392	11.6
II–A–1	1,052	27.2	II–B–1	1,273	17.0	II–C–1	1,356	18.2	II–D–1	1,256	13.4
2	1,446	22.1	2	1,343	18.4	2	1,421	16.0	2	1,507	12.4
3	1,158	23.1	3	1,239	14.9	3	1,342	16.6	3	1,255	12.2
4	1,617	23.5	4	1,450	17.0	4	1,519	15.5	4	1,429	11.8
Total	5,273	23.7	Total	5,305	16.9	Total	5,638	16.5	Total	5,447	12.4
III–A–1	1,268	33.4	III–B–1	1,066	23.7	III–C–1	1,406	16.8	III–D–1	1,139	12.4
2	1,450	25.0	2	1,514	16.4	2	1,197	18.9	2	1,368	13.2
3	1,372	26.0	3	1,460	16.2	3	1,207	17.4	3	1,142	12.7
4	1,406	31.5	4	1,534	18.3	4	1,433	19.8	4	1,479	18.1
Total	5,496	28.8	Total	5,574	18.3	Total	5,243	18.2	Total	5,128	14.3
IV–A–1	1,071	31.1	IV–B–1	1,236	20.3	IV–C–1	1,311	14.8	IV–D–1	1,509	14.6
2	1,421	24.1	2	1,448	15.5	2	1,296	11.2	2	1,273	12.5
3	1,159	24.5	3	1,196	16.6	3	1,476	13.1	3	1,484	11.8
4	1,439	33.0	4	1,354	21.6	4	1,185	15.3	4	1,379	15.1
Total	5,090	28.2	Total	5,234	18.5	Total	5,268	13.5	Total	5,645	13.5
Grand total	21,816	27.2	Grand total	21,637	17.8	Grand total	21,886	14.6	Grand total	21,612	12.9

which the split-plot treatment permits estimating the contributions of outside and inside rows and interactions. Since no substantial differences were found, either in F values or in requirement for significance, between the two bases of calculation, only the comprehensive analysis is shown (table 2).

TABLE 2.—*Analysis of variance of data obtained in the natural crossing study, Knoxville, Tenn., 1939–40*

Variance	Degrees of freedom	Mean square	F		
			Found	Required for—	
				99:1	19:1
Whole plot:					
Barriers, linear	1	1,733.99	116.77	13.74	5.99
Barriers, quadratic	1	232.18	15.64	13.74	5.99
Barriers, cubic	1	17.72	1.19	13.74	5.99
Rows	3	28.72	1.93	9.78	4.76
Columns	3	18.54	1.25	9.78	4.76
Error (a)	6	14.85			
Split plot:					
Outside vs. inside rows	1	138.95	69.47	13.74	5.99
Outside vs. inside rows×barriers, linear	1	40.83	20.41	13.74	5.99
Outside vs. inside rows×barriers, quadratic	1	13.78	6.89	13.74	5.99
Outside vs. inside rows×barriers, cubic	1	.16			
Outside vs. inside rows×rows	3	9.12	4.06	9.78	4.76
Outside vs. inside rows×columns	3	1.22			
Error (b)	6	2.00			
Error (c)	32	2.55			
Total	63	40.21			

As a matter of convenience in interpretation, individual comparisons are tabulated for test conditions and for the interactions of inside vs. outside rows with test conditions (table 2). Variances due to linear, quadratic, and cubic degrees of freedom were calculated from the table of coefficients for orthogonal polynomials of Fisher and Yates (*4*). A study of mean squares for the whole-plot section of the analysis indicates that certain test conditions were effective in reducing the amount of natural crossing. These individual comparisons are based on all of the treatments, so that the no-barrier comparisons as well as the three degrees of guarding are evaluated in the set of individual degrees of freedom shown in table 2. The linear component is decidedly significant, as is indicated by the F value found (116.77), in comparison with 13.74 required for odds of 99:1. The quadratic component barely exceeds odds of 99:1, but indicates that the relationship departs significantly from linearity. An examination of the data reveals that the decrease from no barrier to three rows of corn is proportionately greater than the decreases obtained with successively greater barriers, and this finding suggests that the following specification of individual comparisons is capable of providing further information:

Variance:	Degrees of freedom	Mean square
Barriers, linear (3 vs. 9 rows of corn)	1	202.51
Barriers, quadratic (3 plus 9 vs. 6 rows of corn)	1	5.65
Barriers vs. no barriers	1	1,775.73

In this set of individual comparisons, the linear and quadratic components are estimated on the guarded conditions and the third individual comparison is between all guarded and no barriers. In this set the linear degree of freedom closely approaches odds of 99:1 and the quadratic is not materially different from error. The high contribution for barriers vs. no barriers indicates clearly the differential amount of natural crossing between the unguarded and guarded conditions. The whole-plot section of the analysis, therefore, shows that the use of corn barriers reduces the amount of natural crossing and that the effect of the number of guard rows tends to linearity.

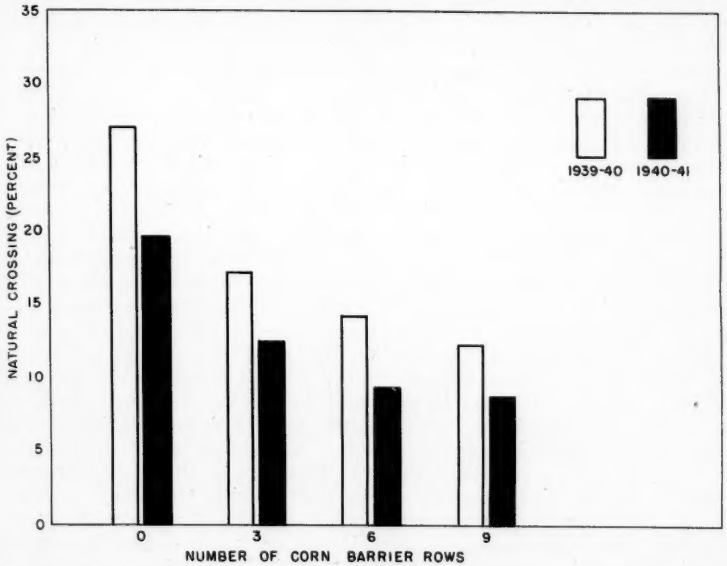

FIGURE 2.—Percentages of natural crossing as averages of four replicates by barrier conditions and years.

Better protection is indicated for plots of cotton surrounded by a larger number of corn rows than for plots surrounded by a smaller number of corn rows, but the means of the various barrier conditions (table 1 and fig. 2) show a proportionately smaller decrease from six- to nine-row than from three- to six-row barriers, and this suggests that an extrapolation of the indicated linearity should not be attempted.

Despite the indicated relative efficiency of different numbers of guard rows, the conclusion must be drawn that the maximum width of corn barriers used in this study was not sufficient to protect pure-line multiplication blocks satisfactorily.

Approximately 10 percent of natural crossing was found with the use of nine-row corn barriers. This amount, or even one-half or one-third as much, in each of the first few generations of multiplication, would largely defeat the objectives of pedigree inbreeding. Better

isolation than was provided by nine rows of corn must be obtained in order to insure a sufficient homozygosity of stocks to permit continued production for several years without the familiar deterioration or "running out" of varieties. The use of corn barriers greatly exceeding nine rows would require a large amount of land if many stocks are being multiplied. Since no evidence is available concerning the minimum number of corn rows that would be required to give adequate protection from natural crossing, it seems more practical to use multiplication blocks isolated by at least one-half mile, and preferably 1 mile, from other cotton varieties. Isolation is difficult to obtain in some sections of the Cotton Belt, but in most cotton States there are localities in which little or no cotton is grown, although climatic and soil conditions are favorable for a fair production. Such localities offer a good opportunity for the economical and safe increase of carefully controlled breeding stocks.

The split-plot section of the analysis (table 2) shows the variance for inside vs. outside rows and interactions for the 1939–40 study. The mean square for outside vs. inside rows is highly significant, and this indicates that, in this study, the number of natural hybrids was greater on the outside rows of the green plots than on the inside rows. Individual degrees of freedom are tabulated for interactions of outside vs. inside rows with test conditions, and these comparisons show that the relative differential between outside and inside rows was associated with the linear degree of freedom for barriers. An examination of the data shows that this interaction was due largely to a greater differential between the no-barrier treatment and treatments consisting of a large number of guard rows. A logical explanation for this differential may be found in the flight habits of the insects largely responsible for natural crossing. Bumblebees, which appear to be the most active agents in natural crossing, frequently move only short distances between successive visits to flowers, and this fact probably accounts for the higher amount of natural crossing on outside rows of the unguarded plots. Conversely, when the distance between plots is rather great, the insects apparently enter the new plot in a substantially random manner, and this results in less distinction between outside and inside rows.

A graphic presentation of the results by rows for the 1939–40 experiment is shown in figure 1. The position of treatments in the various replicates coincides with the field planting plan, so that figure 1 not only presents the actual data by rows but also serves to show the field arrangement of red-leaf cotton, green-leaf cotton, and corn barriers.

The natural crossing experiment of 1940–41 was similar to that of 1939–40 in all details except that three-row plots were used. The results of the two experiments were so closely comparable that a detailed presentation of the data for 1940–41 will be omitted and only summaries shown. The general level of natural crossing was somewhat lower in the second year, the averages ranging from slightly less than 10 to nearly 20 percent, as is evident in the comparison of barrier effects for the 2 years (fig. 2).

The lower level of natural crossing in the second experiment might be due either to the presence of fewer insects or to a proportionately smaller number of flowers on the red plants. Facilities were not available for accurately comparing insect populations in the two

seasons, and consequently no conclusions may be drawn on this point. In each experiment equal plot areas were planted to green-leaf and red-leaf cotton, and the two kinds were thinned in the same manner, so that closely equivalent plant numbers of the contrasting types reached maturity. It was noted, however, that red plants were somewhat less prolific than green plants in the number of flowers produced in 1939 and considerably less prolific than green plants in 1940. These observations suggest that the percentages of natural crossing found are conservative in each of the years when interpreted as amount of natural crossing expected in multiplication blocks where equal prolificacy might be assumed.

An analysis of variance for the 1940–41 data is shown in table 3. It will be noted that this analysis is in general consistent with that for the preceding year's data; consequently it does not seem necessary to discuss the results in detail, except to mention that outside vs. inside rows failed to reach significance in the second year of the study.

These experiments were designed to measure the natural crossing between red and green phenotypes, but it was not possible to detect crossing among the various green plots. It follows that the amounts tabulated for the various conditions actually represent only a part of the natural crossing which occurred between plots. The study in each year consisted of 16-plot units of green, which were used to measure the natural crossing from 16-plot units of red. If these conditions are considered in relation to a practical use of corn barriers where 32 plots of pedigreed inbred cotton stocks were being multiplied, natural crossing from any of the other 31 plots would constitute contamination, and on this basis it might be estimated that the actual amount of crossing among plots was approximately double the amount detected.

These experiments were not designed to measure sib crossing within plots. On the basis of evidence available that amount of natural crossing is inversely related to distance between the parents, it might be expected that sib crossing within plots would exceed that between plots. However, in pure-line increase it may be expected that the

TABLE 3.—*Analysis of variance of data obtained in the natural crossing study, Knoxville, Tenn., 1940–41*

Variance	Degrees of freedom	Mean square	*F*		
			Found	Required for—	
				99:1	19:1
Whole plot:					
Barriers, linear	1	767.84	111.44	13.74	5.99
Barriers, quadratic	1	148.12	21.50	13.74	5.99
Barriers, cubic	1	1.71			
Rows	3	11.32	1.64	9.78	4.76
Columns	3	41.09	5.96	9.78	4.76
Error (a)	6	6.89			
Split plot:					
Outside vs. inside rows	1	9.86	2.40	13.74	5.99
Outside vs. inside rows × barriers, linear	1	16.88	4.12	13.74	5.99
Outside vs. inside rows × barriers, quadratic	1	1.70			
Outside vs. inside rows × barriers, cubic	1	.05			
Outside vs. inside rows × rows	3	4.75	1.16	9.78	4.76
Outside vs. inside rows × columns	3	.88			
Error (b)	6	4.10			
Error (c)	16	8.17			
Total	47	28.02			

progeny of sib crosses within lines will behave in the same way as the population from self-fertilized seed and that consequently natural crossing within plots would be of no practical consequence in the isolated multiplication of pure lines.

NATURAL CROSSING IN ISOLATED MULTIPLICATION BLOCKS

The need for isolation distances exceeding those practicable with corn barriers, as indicated by results from the 1939–40 study, led to an extension of the experiment to include growing small blocks of pure red-leaf cotton on adjacent farms at known distances from green-leaf cotton at the station. The distance and direction from the station for each of the six blocks of pure red-leaf cotton grown in 1941, and the percentage of natural crossing found for each, are shown in figure 3.

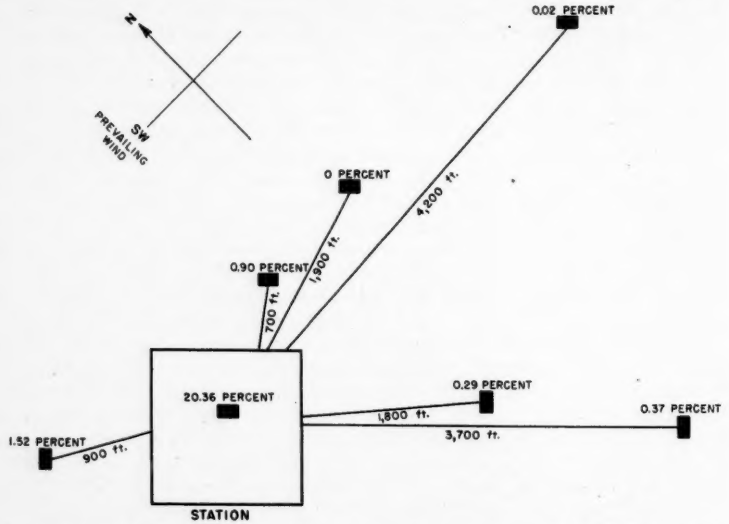

FIGURE 3.—Distance and direction from green-leaf cotton of isolated blocks of red-leaf cotton with percentages of natural crossing.

Seed produced in each of the isolated blocks was grown in 1942, and the plants were classified for color. The total number of plants, the number of hybrid plants, and the percentage of natural crossing are shown in table 4. In addition to the off-station isolated red blocks, a station planting of adjacent red and green rows was included as a check on the general level of natural crossing during the 1941 growing season. A test planting of red selfed seed was grown and classified with the other lots in 1942 as a check on the homozygosity of the red stocks used in planting the 1941 isolated blocks.

It will be noted that the amount of natural crossing is not entirely consistent with the distance of isolation. The 700-foot and 900-foot plantings were separated from the station by open fields of corn and grassland. Small patches of timber grew between the station and the other plantings, and these, together with the rolling topography,

TABLE 4.—*Natural crossing in isolated blocks, Knoxville, Tenn., 1941*

Location of red cotton	Distance from green cotton	Total plants	Hybrid plants	
	Feet	*Number*	*Number*	*Percent*
Station	(1)	3,089	629	20.36
Isolated block:				
A	900	3,617	55	1.52
B	700	3,223	29	.90
C	1,900	5,333	0	0
D	4,200	4,336	1	.02
E	1,800	3,777	11	.29
F	3,700	3,528	13	.37
Check (selfed)		3,978	0	0

[1] Station planting of adjacent red and green rows.

undoubtedly introduced certain inequalities in the relation of distance to amount of natural crossing.

These data indicate that the cotton breeder should decide on the amount of natural crossing that may be tolerated in a particular program of multiplication and distribution, and choose isolation distances accordingly. The genetic homozygosity of the parental stocks to be increased is a primary consideration in deciding on the amount of natural crossing that may be tolerated. If stocks have been inbred 5 years or more and seem promising enough to warrant long-time production, it seems logical that each stage of multiplication should be isolated sufficiently to maintain the genetic purity.

NOTES AND OBSERVATIONS ON INSECTS

It was not possible to make extensive studies on insect populations, but a collection was made during a few short periods in the field. No attempt was made to capture all the insects on the cotton flowers, but rather to get one or a few of each of the species observed. A list of the insects found on cotton flowers follows.[3]

Insects most probably concerned in transfer of pollen:

	Common name
Bombus spp. (Bombidae)	Bumblebee.
Melissodes obliqua Say? (Anthophoridae)	Soil-nesting bee.
Melissodes spp. (Anthophoridae)	Do.
Halictus ligatus Say (Halictidae)	Do.
Halictus (Chloralictus) spp. (Halictidae)	Do.
Augochlorella spp. (Halictidae)	Do.
Coleomegilla maculata (Degeer) (Coccinellidae)	Ladybeetle.
Diabrotica duodecimpunctata (F.) (Chrysomelidae)	Spotted cucumber beetle.
Epicauta trichrus (Pallas) (Meloidae)	Blister beetle.

Insects less probably concerned in transfer of pollen:

Formica pallidefulva schaufussi Mayr (Formicidae)	Pale-yellow ant.
Dexiini, genus and species uncertain (Tachinidae)	Tachinid fly.
Olla oculata (Say) (Coccinellidae)	Ladybeetle.
Cycloneda munda (Say) (Coccinellidae)	Do.
Hippodamia convergens Guér. (Coccinellidae)	Convergent ladybeetle.
Centrinaspis sp. (Curculionidae)	Weevil.
Acrosternum hilare (Say)	Green stinkbug.

[3] Identification made through courtesy of Dr. C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture.

Insects less probably concerned in transfer of pollen—Continued:

Podisus maculiventris (Say)	Spined soldier bug.
Geocoris punctipes (Say)	Big-eyed bug.
Lygus oblineatus (Say)	Tarnished plant bug.
Gypona sp., nymph (Homoptera, Cicadellidae).	Leafhopper.
Diabrotica vittata (F.) (Chrysomelidae)	Striped cucumber beetle.
Colaspis sp., near *brunnea* (F.) (Chrysomelidae).	Leaf beetle.
Collops quadrimaculatus (F.) (Malachiidae)	Malachiid beetle.
Lebia sp. (Carabidae)	Ground beetle.
Psammocharidae (not further determinable)	Spider wasp.

The separation of the insects into groups more and less probably concerned in natural crossing was made on the basis of feeding habits and other characteristics. Field observations indicate that bumblebees were the most numerous and active insects visiting cotton flowers. Honeybees were observed but not captured, and therefore are not included in the foregoing list. Other insects were present, but the various species of bees were the only insects that were observed to make rapid and rather systematic visits to the flowers. Movement of the bees within the plot usually was only a few feet at a time and from plant to plant along the row. Flight from the plot was usually through the corn barriers rather than up and over the top of the corn.

PRODUCTION OF HYBRID SEED FOR COMMERCIAL PLANTING

At various times the question has been raised whether a practical and inexpensive technique could be developed to produce hybrid cotton seed for commercial plantings. Artificial crossing is too expensive to be profitable on the basis of increases in yield usually expected for hybrid over other stocks within a species. However, in some species crosses, such as sea island × upland (*Gossypium barbadense* × *G. hirsutum*), certain combinations of lines usually give a marked increase in yield over the sea-island parent together with fiber length similar to sea island. These increases depend to a considerable degree on the specific lines used in crossing; and more work in identifying the best lines, studying the technique of producing crosses, and solving certain ginning problems is needed before hybrid production may be recommended.

In the present study the area of land in green- and red-leaf cotton was the same, and the stand was substantially equal, but flower production in the green plots was materially better than in the red. It follows that, despite care in designing the experiment, absolute equality in opportunity was not achieved, and consequently the 20 to 25 percent of natural crossing found in the no-barrier plots is undoubtedly conservative for the conditions under which the tests were made.

Maximum cross-pollination would be expected in areas having large insect populations and in fields near pastures, meadows, or wasteland. In the commercial production of natural crosses it would be logical to plant alternate rows or alternate hills of the contrasting parental stocks, since such an arrangement might be expected to give a larger percentage of hybrids than would occur in adjacent block plantings, such as were used in this study. Seed produced by both parents could be used for planting, if the hybrids are clearly distinguishable in the seedling stage. Any contrasting pair of characters exhibiting inter-

mediate dominance of the 1:2:1 ratio type may be used if the intermediate class is easily distinguishable in the seedling stage. Hand thinning would be required, and the characters used should exhibit marked contrast, so that field workers could be trained to easily recognize and accurately remove the homozygous types.

If the amount of seed planted, including natural crosses, is five to eight times the desired number of plants in the final stand, it might be expected that the thinning-out of all plants, except intermediate ones, would give a satisfactory stand. With hill-dropping 18 to 24

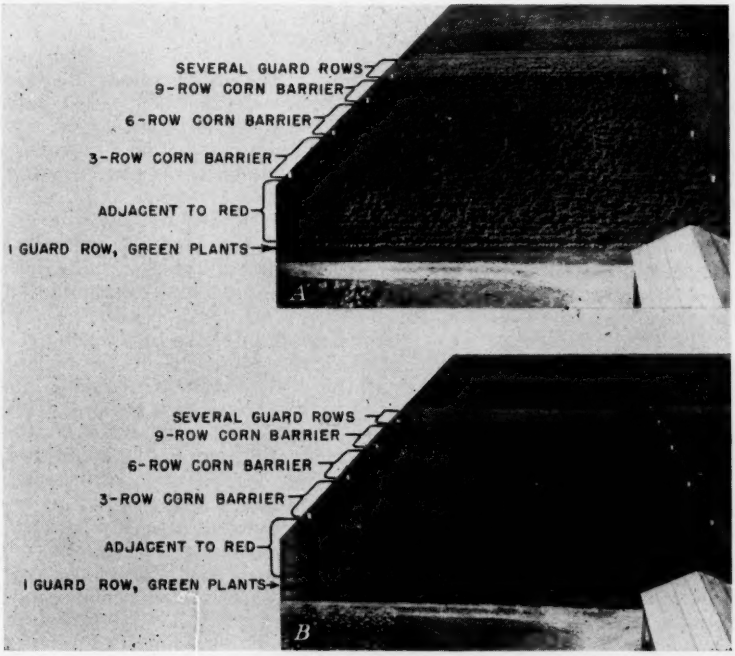

FIGURE 4.—Field views of natural crosses, showing the proportionate stand from the various conditions of guarding: *A*, June 27; *B*, August 22.

inches apart and hand thinning, a hybrid crop might be planted with approximately the same amount of seed ordinarily used when conventional planting and chopping practices are followed.

Figure 4 illustrates the feasibility of obtaining stands when using natural-cross hybrids. Both field views are of the 1941 testing area, showing the appearance of the block on June 27 (*A*) and on August 22 (*B*). The rate of planting of each lot was approximately 1½ bushels per acre. The plants in the area bounded by white stakes are all intermediate red with the exception of one border row next to the roadway in the foreground. This block was used in 1941 for planting the 1940 seed produced on green plots, and, in thinning, all green plants were removed and the intermediate red plants were permitted to remain. The sections are arranged in order of barrier treatment. The first 12

rows from the foreground border to the first pair of white stakes are from the adjacent red-green plots, and it will be noted that the stand is reasonably satisfactory. The successive blocks of 12 rows, in the direction of the background, are from the plots protected by the three-, six-, and nine-row corn barriers, respectively, and in these sections the stand decreases progressively in proportion to the amount of natural crossing. Figure 4 shows the results for the year in which the rate of natural crossing was lowest, but illustrates the conclusion that the production of naturally crossed seed, for use in commercial plantings, appears feasible.

SUMMARY AND CONCLUSIONS

A study was conducted for 2 years on the effectiveness of guard plantings of corn on natural crossing in cotton, in which four conditions of guarding were included: (1) Green-leaf adjacent to red-leaf cotton surrounded by corn; (2) green-leaf separated from red-leaf by three rows of corn; (3) green separated from red by six rows of corn; and (4) green separated from red by nine rows of corn.

The amount of natural crossing was determined by planting approximately 1,500 seeds from each row of the plots of green-leaf cotton and determining the percentage of natural hybrids. The data may be interpreted simply and directly, since equal areas were planted to green- and to red-leaf cotton.

The results indicate that corn barriers are effective in reducing the amount of natural crossing and that the reduction tends toward linearity for the different barrier widths used in this experiment.

Despite the indicated efficiency of barriers in reducing the amount of natural crossing, the results show clearly that the barrier widths of corn used did not afford sufficient protection, under the conditions of this experiment, for the multiplication of selfed-line seed stocks. The minimum amount of natural crossing found would in a few generations of multiplication reduce the homozygosity to a point where the seed stocks would be too badly mixed for continued production.

Small-block plantings of red-leaf cotton, made at distances ranging from 700 to 4,200 feet from green-leaf cotton, established the occurrence of natural crossing at distances up to 0.8 mile. It therefore seems clear that distances of 1 mile or more will be required to provide complete isolation, under the conditions prevailing in this study.

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